Increasing resistance to fluoroquinolones in *Escherichia coli* from urinary tract infections in The Netherlands

W. Goettsch\textsuperscript{a}, W. van Pelt\textsuperscript{a}, N. Nagelkerke\textsuperscript{a}, M. G. R. Hendrix\textsuperscript{b}, A. G. M. Buiting\textsuperscript{c}, P. L. Petit\textsuperscript{d}, L. J. M. Sabbe\textsuperscript{e}, A. J. A. van Griethuysen\textsuperscript{f} and A. J. de Neeling\textsuperscript{a}

\textsuperscript{a}National Institute of Public Health and the Environment, PO Box 1, 3720 BA Bilthoven and Public Health Laboratories at \textsuperscript{b}Enschede, \textsuperscript{c}Tilburg, \textsuperscript{d}Rotterdam, \textsuperscript{e}Goes and \textsuperscript{f}Nijmegen, The Netherlands

In continuous surveillance of routine samples from five Dutch laboratories, we studied resistance to the antibiotics most commonly prescribed for urinary tract infections (UTI) in The Netherlands, namely norfloxacin, amoxycillin, trimethoprim and nitrofurantoin, from 1989 to 1998 in >90 000 *Escherichia coli* isolates. Resistance to norfloxacin increased from 1.3% in 1989 to 5.8% in 1998. Multiresistance, defined as resistance to norfloxacin and at least two of the other three antibiotics, increased from 0.5% in 1989 to 4.0% in 1998. Multivariate analysis of the norfloxacin resistance demonstrated that this yearly increase (the odds ratio was 1.0 in 1989, 1.6 in 1992, 2.9 in 1995 and 6.1 in 1998) was independent of other determinants of resistance to norfloxacin, such as age, gender and origin of the isolate. Analysis of strata, classified by year, age and gender, demonstrated an association between prescription of fluoroquinolones (defined daily doses per case of UTI) and resistance to norfloxacin in *E. coli* (*P* < 0.001). There was no significant association with the prescription of nitrofuran derivatives (nitrofurantoin) and trimethoprim with or without sulphanemethoxazole. The yearly increase of resistance to fluoroquinolones in *E. coli* from UTI may stem from increased prescription of fluoroquinolones for UTI. Resistance of *E. coli* to these agents is likely to increase further as fluoroquinolone use increases in future.

**Introduction**

Increasing resistance to amoxycillin and trimethoprim in *Escherichia coli*, the main causative pathogen of urinary tract infections (UTI), has been demonstrated in urinary tract isolates obtained from patients visiting their general practitioners (GPs).\textsuperscript{1-5} Additionally, findings from routine testing by medical microbiological laboratories indicate growing resistance to several antibiotics in *E. coli* from urinary tract isolates.\textsuperscript{3} In The Netherlands, the Dutch College of General Practitioners recommend a 3 day course of trimethoprim or nitrofurantoin for the treatment of UTI.\textsuperscript{4,6,7} However, in the past few years, fluoroquinolones have been prescribed more frequently for the treatment of such infections. This may have led to an increase in fluoroquinolone-resistant *E. coli* infections, which are difficult to treat.

In this article we report on the development of resistance to fluoroquinolones in *E. coli* isolates from UTI in The Netherlands. Data from a sentinel surveillance programme, which collected data on resistance from five laboratories from 1989 to 1998, were combined with data on prescription of fluoroquinolones in the community in order to investigate whether patterns of resistance correlate with trends in the prescription of these antibiotics.

**Materials and methods**

**Data sampling and processing**

During the study period, 1989–98, five regional public health laboratories in The Netherlands (Rotterdam, Nijmegen, Goes, Tilburg and Enschede) forwarded all routine data on the occurrence of *E. coli*, including antibiotic susceptibility data, to the National Institute of Public Health and the Environment. These laboratories cover approximately 14% of the Dutch population (15.4 million inhabitants in 1994). They tested >75% of the *E. coli* isolates for resistance to amoxycillin, trimethoprim, nitro-
furantoin and norfloxacin. Only *E. coli* isolates from urine were included. Repeat isolates of *E. coli* from the same patient taken within 2 months were ignored. Detailed information on the number of prescriptions and defined daily doses (DDDs) prescribed in the community (including nursing homes and outpatient clinics; excluding inpatient clinics) was obtained for fluoroquinolones, nitrofurantoin derivatives, trimethoprim and co-trimoxazole (sulphamethoxazole and trimethoprim) from the Drug Information Project of the Supervising Board for Health Care Insurance in The Netherlands, Amstelveen (1992–97). Information on the incidence of UTI was obtained from a sentinel GP surveillance system in the east of The Netherlands (Nijmegen and surroundings).

The incidence of UTI was used to investigate whether there was a link between the number of prescriptions and the incidence of resistance on the basis of groups stratified by age and gender. Data from a sentinel GP surveillance system indicated that the incidence of UTI was lower in younger age groups than in older age groups, resulting in a relatively low prescription rate of antibiotics for treatment of UTI. So, in order to link prescription to resistance directly, the number of prescriptions (DDD/1000 medically insured) was divided by the incidence (UTI/1000 person-years), giving the number of prescriptions per case of UTI (DDD/case UTI).

**Laboratory methods**

Susceptibility test results were interpreted with reference to breakpoints published by the Dutch Committee on Susceptibility Testing Guidelines. The breakpoints for resistance were: $\geq 64 \text{ mg/L}$ for amoxycillin and nitrofurantoin, and $\geq 16 \text{ mg/L}$ for trimethoprim and norfloxacin. Sensitivity testing methods were not standardized.

**Statistical methods**

Analyses were performed with SAS software, version 6.12 (SAS Institute, Cary, NC, USA). The percentage of norfloxacin-resistant *E. coli* was univariately compared for independent variables, such as year of isolation, age of patient, source of isolate (laboratory), origin of the patient (general practice, nursing home, inpatient or outpatient clinic). Using multivariate, logistic regression, the relative risks for the dependent variable, resistance to norfloxacin, were estimated for the independent variables.

Because of cross-resistance between norfloxacin and other fluoroquinolones used for therapy of UTI, such as ciprofloxacin and ofloxacin, it is justifiable to compare norfloxacin resistance (as a marker for fluoroquinolone resistance) with the total prescription of fluoroquinolones. Separate strata for year of isolation, gender and age were used to study the associations between resistance to norfloxacin (excluding samples from inpatient clinics) and prescription of fluoroquinolones, nitrofurantoin derivatives and trimethoprim with or without sulphamethoxazole.

Linear regression curves were fitted using prescription rate as the independent variable and resistance as the dependent variable. The prescription rate, measured in DDD, was adjusted for the incidence of urinary tract infection in the different age groups (averaged for 6 years (1992–97) for every age group), resulting in DDD/case UTI.

**Results**

**Proportion of resistant *E. coli* strains**

A total of 91 669 *E. coli* isolates were available for analysis; of these, 2232 were resistant to norfloxacin, 23 226 to trimethoprim, 4726 to nitrofurantoin and 31 903 to amoxycillin. Resistance to trimethoprim and norfloxacin had increased steadily over the study period, whereas resistance to amoxycillin and nitrofurantoin had remained constant (Table I). The increase in norfloxacin resistance was associated with an increase in multiresistance, defined as resistance to norfloxacin and to at least two of the other three antibiotics (Table I).

The trend towards an increase in norfloxacin resistance was found in all five participating laboratories (Figure 1a). The increase in norfloxacin resistance was most pronounced in *E. coli* isolates from general practice, outpatient clinics and nursing homes (Figure 1b). Resistance to norfloxacin increased in both males and females (Figure 1c). Age appeared to influence the development of resistance to norfloxacin over time: resistance remained low in the youngest age group (<15 years), but increased most steeply in the oldest age group (>74 years) (Figure 1d).

**Multivariate analysis of determinants of norfloxacin resistance**

Multivariate analysis showed that the increase in norfloxacin resistance over time was independent of other risk factors, such as age, gender, laboratory and origin of the patient. The effects of age, gender and patient’s origin on norfloxacin resistance were not influenced by the location of the laboratories (Table II). However, the effect of age on resistance was strongly determined by gender; in male patients, resistance to norfloxacin was already high in younger age groups, in contrast to the corresponding age groups in female patients (Table II).

**Developments in the prescription of fluoroquinolones, nitrofurantoin and trimethoprim/co-trimoxazole in The Netherlands**

Data from the Drug Information Project of the Supervising Board for Health Care Insurance in The Netherlands (GIP/CVZ), Amstelveen, indicated that the prescription of fluoroquinolones had doubled from 1990 onwards (Table III). In contrast, the prescription of nitrofurans...
Fluoroquinolone resistance in *Escherichia coli*

Table I. Ten-year development of resistance to four antibiotics in *E. coli* from urinary tract isolates (data from five Dutch regional laboratories covering approximately 14% of the Dutch population)

<table>
<thead>
<tr>
<th>Year</th>
<th>Norfloxacin no. of isolates</th>
<th>% (no.) resistant</th>
<th>Nitrofurantoin no. of isolates</th>
<th>% (no.) resistant</th>
<th>Trimethoprim no. of isolates</th>
<th>% (no.) resistant</th>
<th>Amoxycillin no. of isolates</th>
<th>% (no.) resistant</th>
<th>Multiresistant no. of isolates</th>
<th>% (no.) resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>4147</td>
<td>1.3 (54)</td>
<td>4176</td>
<td>6.4 (266)</td>
<td>4173</td>
<td>21.6 (902)</td>
<td>4176</td>
<td>29.4 (1227)</td>
<td>4134</td>
<td>0.5 (22)</td>
</tr>
<tr>
<td>1990</td>
<td>8974</td>
<td>1.1 (98)</td>
<td>8983</td>
<td>5.7 (514)</td>
<td>8986</td>
<td>23.4 (2102)</td>
<td>8997</td>
<td>35.0 (3143)</td>
<td>8955</td>
<td>0.6 (57)</td>
</tr>
<tr>
<td>1991</td>
<td>11309</td>
<td>1.4 (158)</td>
<td>11325</td>
<td>4.5 (511)</td>
<td>11317</td>
<td>23.0 (2599)</td>
<td>11332</td>
<td>35.3 (4003)</td>
<td>11293</td>
<td>0.7 (76)</td>
</tr>
<tr>
<td>1992</td>
<td>11390</td>
<td>1.5 (169)</td>
<td>11409</td>
<td>4.9 (559)</td>
<td>11391</td>
<td>24.3 (2769)</td>
<td>11418</td>
<td>33.6 (3841)</td>
<td>11366</td>
<td>1.1 (117)</td>
</tr>
<tr>
<td>1993</td>
<td>10231</td>
<td>1.7 (172)</td>
<td>10250</td>
<td>4.7 (477)</td>
<td>10248</td>
<td>24.8 (2543)</td>
<td>10258</td>
<td>33.6 (3447)</td>
<td>10223</td>
<td>1.2 (121)</td>
</tr>
<tr>
<td>1994</td>
<td>9875</td>
<td>2.1 (208)</td>
<td>9943</td>
<td>4.8 (472)</td>
<td>9939</td>
<td>25.7 (2553)</td>
<td>9948</td>
<td>33.7 (3354)</td>
<td>9867</td>
<td>1.4 (137)</td>
</tr>
<tr>
<td>1995</td>
<td>10077</td>
<td>2.8 (279)</td>
<td>10079</td>
<td>4.8 (483)</td>
<td>10082</td>
<td>26.9 (2716)</td>
<td>10090</td>
<td>35.6 (3594)</td>
<td>10054</td>
<td>1.8 (144)</td>
</tr>
<tr>
<td>1996</td>
<td>9239</td>
<td>3.3 (307)</td>
<td>9240</td>
<td>5.7 (526)</td>
<td>9242</td>
<td>26.9 (2482)</td>
<td>9260</td>
<td>35.7 (3307)</td>
<td>9230</td>
<td>2.3 (210)</td>
</tr>
<tr>
<td>1997</td>
<td>9402</td>
<td>4.1 (383)</td>
<td>9420</td>
<td>5.2 (490)</td>
<td>9417</td>
<td>28.0 (2632)</td>
<td>9426</td>
<td>36.7 (3455)</td>
<td>9393</td>
<td>2.8 (263)</td>
</tr>
<tr>
<td>1998</td>
<td>7025</td>
<td>5.8 (404)</td>
<td>7020</td>
<td>6.1 (428)</td>
<td>7015</td>
<td>27.5 (1928)</td>
<td>7025</td>
<td>36.0 (2532)</td>
<td>7011</td>
<td>4.0 (281)</td>
</tr>
</tbody>
</table>

*Multiresistant is defined as resistant to norfloxacin and at least two of the other three antibiotics (amoxycillin, trimethoprim and nitrofurantoin).

Figure 1. Proportions of norfloxacin-resistant strains according to (a) laboratory (the five laboratories are represented by different symbols and line styles), (b) origin (•, general practice; ●, nursing home; □, outpatient clinic; ▲, inpatient clinic). (c) gender (●, female; ○, male) and (d) age group (for female patients only) (○, <15 years; □, 15–24 years; ▲, 25–44 years; ●, 45–64 years; •, 65–74 years; ■, >74 years).
derivatives (mainly nitrofurantoin), and trimethoprim with or without sulphamethoxazole had decreased in this period (Table III).

Linear regression of the association between prescription rate in DDD/case UTI for fluoroquinolones, nitrofuran derivatives and trimethoprim/co-trimoxazole and resistance to norfloxacin revealed a significant association only between norfloxacin resistance and the prescription of fluoroquinolones ($P = 0.002$). Prescription of nitrofuran derivatives ($P = 0.1$) and trimethoprim with or without sulphamethoxazole ($P = 0.3$) was not significantly associated with norfloxacin resistance. Figure 2 depicts the relationship between norfloxacin resistance and fluoroquinolone usage.

**Discussion**

This study indicates clearly that norfloxacin resistance in *E. coli* isolates is increasing. However, the absolute proportion of resistant strains must be interpreted with caution. Results from routine bacteriological testing from general practice tend to overestimate the resistance in this section of the patient population because the submission of specimens tends to be selective (possibly following the failure of empirical treatment). In a previous study from our centre, trimethoprim resistance in *E. coli* isolates routinely sent by GPs was more than twice that in isolates obtained from patients who had not been treated with antibiotics for at least 6 months. Comparison of our results with findings
Fluoroquinolone resistance in *Escherichia coli* shows a similar difference in the proportion of resistant strains from previous studies on isolates of unselected patients. Nevertheless, an increase in resistance, as was demonstrated in our study, may be predictive of developments in the community, provided the results of the disc tests in local laboratories and MIC data show good agreement. In such a case, as was recently suggested for *E. coli*, small changes in resistance, which may be detected more easily when sample sizes are large, show a potential for the emergence of an actual resistance problem. However, we cannot exclude the possibility that this multiple resistance developed from selection of genetically linked resistance genes.

The incidence of resistance differed among laboratories. Partly owing to different testing methods, the trend towards increasing resistance was demonstrated in laboratories that used the most common techniques. The increased resistance in older age groups (mainly female) might result from increased cumulative exposure to fluoroquinolones in older age groups. The increased resistance in female patients has been previously documented. The increased resistance in female patients can be explained by the different nature of UTIs in males and females. In female patients, uncomplicated cystitis is more common, whereas in male patients complicated UTIs are more common. Males with UTIs complicated by prostatitis are more common, whereas in females with prostatitis, uncomplicated cystitis is more common.

### Table III. Prescription in The Netherlands of fluoroquinolones (FQ), nitrofuran derivatives (NIT) and trimethoprim/co-trimoxazole (TMX) from 1990 to 1997 (total), and age- and gender-specific for fluoroquinolones (1992–97). Prescription is expressed in defined daily doses/1000 medically insured. Data were obtained from the Drug Information Project of the Supervising Board for Health Care Insurance in The Netherlands (GIP/CVZ), Amstelveen.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total</th>
<th>Males receiving quinolone</th>
<th>Females receiving quinolone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>286.7</td>
<td>351.1</td>
<td>153.5</td>
</tr>
<tr>
<td>1991</td>
<td>256.1</td>
<td>357.9</td>
<td>172.2</td>
</tr>
<tr>
<td>1992</td>
<td>265.4</td>
<td>375.8</td>
<td>179.7</td>
</tr>
<tr>
<td>1993</td>
<td>262.3</td>
<td>370.6</td>
<td>204.6</td>
</tr>
<tr>
<td>1994</td>
<td>260.6</td>
<td>350.3</td>
<td>232.3</td>
</tr>
<tr>
<td>1995</td>
<td>257.7</td>
<td>323.2</td>
<td>256.8</td>
</tr>
<tr>
<td>1996</td>
<td>246.3</td>
<td>318.4</td>
<td>276.9</td>
</tr>
<tr>
<td>1997</td>
<td>243.0</td>
<td>303.5</td>
<td>297.0</td>
</tr>
</tbody>
</table>
lative lifetime exposure in the older patients, or to a more complicated nature of the UTI more obviously warranting prescription of fluoroquinolones, or to the recent increase in fluoroquinolone prescription, especially for older patients.

Currently there are no indications that fluoroquinolones are prescribed regularly for respiratory infections in The Netherlands. Thus, this group of antibiotics is still most often used for the therapy of UTI. Prescription of fluoroquinolones nearly doubled from 1990 to 1997. In contrast, prescription of nitrofuran derivatives (mainly nitrofurantoin) and trimethoprim/co-trimoxazole decreased over the study period. This indicates that not only GPs, but also physicians in nursing homes and outpatient clinics, are prescribing fluoroquinolones for UTI increasingly frequently. However, it is not clear whether they prescribe these antibiotics as first-, second- or third-choice for UTI.

A comparison of the trend in norfloxacin resistance and the rates of prescription for fluoroquinolones, nitrofuran derivatives and trimethoprim/co-trimoxazole indicated a significant relationship only between norfloxacin resistance and fluoroquinolone prescription. This suggests that increased prescription of fluoroquinolones may be the driving selection force leading to increased norfloxacin (fluroquinolone) resistance in E. coli in the community. A recent case–control study showed that increased resistance to trimethoprim in Gram-negative bacteria isolated from urine samples is associated primarily with the increased use of this antibiotic and not with the use of other antibiotics. In two other hospital case–control studies, resistance to fluoroquinolones in Gram-negative bacteria from nosocomial infections was strongly associated with prescription of this group of antibiotics. In these studies, too, associations with the use of other antibiotics were not significant. Thus, our study indicates that resistance to norfloxacin in the community is only associated with the prescription of fluoroquinolones, and not with that of other antibiotics.

Acknowledgements

The authors are obliged to J. F. Piepenbrink and R. J. van der Vaart from the Drug Information Project of the Supervising Board for Health Care Insurance in The Netherlands (GIP/CVZ), Amstelveen, for providing data on the antibiotic prescribing Board for Health Care Insurance in The Netherlands. The authors are obliged to J. F. Piepenbrink and R. J. van der Vaart from the Drug Information Project of the Supervising Board for Health Care Insurance in The Netherlands.

References


Received 20 October 1999; returned 20 January 2000; revised 18 February 2000; accepted 27 March 2000